

Immunofluorescence SFTPC, SCGB1A1, and ACTA2

Purpose: Stain slides of 7µm frozen sections of P03 C57BL6 lung for **SFTPC**, **SCGB1A1**, and **ACTA2** with antigen retrieval.

Day 1

1. For Frozen tissue, rinse 2X in PBS, then 5 min in 4% PFA/PBS, then rinse 1X in PBS for 5 min.
2. Briefly equilibrate slides in Antigen Retrieval Buffer.
3. Antigen retrieval, pH 6.0 (times will vary according to microwave).
4. 10 mM sodium citrate, pH 6.0, and heat in a microwave at 96°C. *We usually do a series of three runs (6-7 minutes each run) to equal the time/temp because of evaporation (refill coplin jars with dH₂O).
5. Microwave according to instructions on microwave.
6. Cool on countertop, 15 min.
7. Rinse with dH₂O.
8. 1X PBS, 5 min.
9. Block in 4% Goat serum/PBS-T, 2 hours at RT.
10. For Rabbit anti-**SFTPC** (RB458, BL 8/24/11, in house) dilute 1:1000, For guinea pig anti-**SCGB1A1** (G210, BL 3/24/11) dilute 1:1500, for mouse anti-**ACTA2** (**α Smooth muscle actin**, A5228, Sigma) dilute 1:2000 in blocking buffer. Spin down in µfuge for 10 minutes and incubate on tissue overnight @ 4°C.

Day 2

1. Rinse slides in PBS-T 3X, 5 min.
2. Apply secondary antibody, Goat Alexa Fluor 488 anti-rabbit IgG (**A11034**, Lot# 1298480, for anti-**SFTPC**), Goat Alexa Fluor 555 anti-guinea pig IgG (**A21435**, Lot# 1571699, for anti-**SCGB1A1**) at 1:200, Donkey Alexa Fluor 647 anti-mouse IgG **A31571**, Lot# 1549801, **ACTA2**) at 1:200, in blocking buffer. Spin down in µfuge for 10 min, apply to tissue and incubated at room temperature for 1 hour.
3. Rinse in PBS-T 3X, 5 min.
4. Dilute DAPI 1:2000 and apply to slides for 10 min.
5. Wash in PBS-T 3X, 5 min.
6. Rinse slides in 0.1M PB or TB, 3X, 5 min.
7. Add 1 drop of Prolong Gold anti-fade mounting medium (P36930).
8. Coverslip with Gold Seal Coverslip (Cat# 3422 Electron Microscopy Sciences, 22 X 22 mm).
9. Allow Prolong Gold to cure overnight at room temp in light sealed box.
10. Store slides in light-sealed box at 4°C.

Tissue Used: LMM.16.83A.4.23
PND03 C57BL6
Gender: Male
Weight= 2.1g

Appendix

Antigen Retrieval Solution

18ml Solution A- 0.1M Citric Acid (Sigma, C1909) pH 2.5
82ml Reagent B- 0.1M Sodium Citrate (Sigma, S4641) pH 8.2
1L dH₂O
pH 6.0